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MILK ALLERGY[1]

JOSEPH R. SPIES
Dairy Products Laboratory, Agricultural Research Service
U.S. Department of Agriculture, Washington, D.C. 20250

ABSTRACT

A general review of milk allergy and a summary of current research on milk at Dairy Products Laboratory (DPL) is presented. Milk allergy occurs primarily in infants and children under 2 years of age. It became more prevalent in the U.S. as breast feeding declined and feeding of cow's milk increased. Milk allergy (atopic and anaphylactic) has an immunological basis as distinguished from such diseases as lactose intolerance and galactosemia. The reported incidence of milk allergy varies widely from 30% in allergic children to 0.1 to 7% in nonallergic children. Symptoms of milk allergy are asthma, rhinitis, vomiting, abdominal pain, diarrhea, urticaria, and anaphylaxis. Crib deaths have been attributed to milk allergy. Prognosis is that milk allergy usually disappears by age 2. Milk proteins are the etiological agents in milk allergy. Milk contains from 12-14 immunologically distinguishable proteins, all of which are potential allergens. DPL is doing basic research on milk allergens to elucidate the mechanism of the allergic response to ingested milk. Demonstration of new antigens (potential allergens) generated by brief pepsin hydrolysis of four milk proteins-casein, α-lactalbumin, β-lactoglobulin and bovine serum albumin, is the basis for a new concept of the role of digestion products in immediate type milk and food allergy.

A distinguished allergist at Massachusetts General Hospital stated in 1950 that, "There is perhaps no field of medicine in which more divergent views are held than in that of allergy to foods" *(34)*. A search of the literature on milk allergy reveals that, although much research has been done since that time, it appears that this opinion is still valid. The literature on milk and food allergy has been amply reviewed *(5, 8, 9, 10, 13, 14, 20, 26, 32, 33)*.

The term "allergy" was first used by von Pirquet *(51)* in 1906 to denote an altered capacity of a human to react to a second injection of horse serum. Since that time allergy generally has been used to describe all forms of hypersensitivity in man. An allergen may be defined as an ordinarily harmless substance present in the diet or environment, able to produce such human diseases as asthma, hay fever, eczema, and gastrointestinal upsets upon contact with a previously sensitized person.

Similarly, Portier and Richet *(38)* in 1902 first used the term "anaphylaxis" to describe sensitization and later shock when a foreign protein (antigen) was injected into an animal.

[1]Presented at the 59th Annual Meeting of International Association of Milk, Food, and Environmental Sanitarians, Milwaukee, Wisconsin, August 21-24, 1972 under the title: "Dairy Food Allergens and Allergy."

Studies of allergy and anaphylaxis have been closely linked because there is a basic relationship between them although they do have important distinguishing differences. Both involve release of histamine or histamine-like substances when allergen or antigen comes in contact with specific antibodies (immunoglobulins) in various shock tissues thereby producing characteristic reactions. By definition milk allergy is the body response to an allergen-antibody reaction. There are three types of allergy, namely, atopic immediate type in which symptoms appear in a few up to 60 min; atopic delayed type in which symptoms appear in 1 to 36 hr although delays of up to several days have been reported; anaphylactic type in which symptoms are violent, sometimes fatal, and which may occur in seconds to a few minutes.

Not all symptoms which arise on ingestion of milk are caused by milk allergy. There are several nonimmunological reactions to ingestion to milk which resemble allergy such as lactose intolerance *(39)*, galactosemia, and bacterial contamination. Also several foreign substances in milk cause immunological reactions. Among those reported are ragweed tops, linseed, cottonseed, wheat, peanut, and penicillin *(9)*. Penicillin became important enough to require legal restriction on the use of milk for a period of time after injection of penicillin into the udder. It was generally assumed that there is enough penicillin in milk from treated cows to cause allergic reactions in sensitive persons but probably not enough to sensitize persons not already sensitive *(9)*.

INCIDENCE OF MILK ALLERGY

The reported incidence of bovine milk allergy ranges from < 0.1% to 30% *(9)*. The incidence depends on the group selected and other criteria. The highest value was 30% in a group of allergic children. In a group designated "nonallergic children," the incidence was 0.3%. There is, as to be expected, considerable variation in incidences in similarly designated groups reported by different workers. Thus in four groups designated as "all children," the incidences were from < 0.1 to 7%. In a group designated "well babies" the incidence was 1%. This wide variation reflects diagnostic difficulties in selection of the groups. At present there is no objective laboratory test available that has more than contributory value in diagnosis. Diagnosis is made by clinical evalu-

ation which is difficult because of the multiplicity of symptoms and that many of these symptoms result from other causes. The provocative test, to be described below, at best, involves some subjective evaluation by the physician as well as the ever-present possibility that patients do not follow elimination diets either accidentally or wilfully.

Diagnosis of Milk Allergy

Diagnosis of milk allergy is difficult not only because of the multiplicity of symptoms which are duplicated in other diseases but also because of the lack of clear-cut objective tests. Another complicating factor is the widely varying degrees of sensitivity ranging from a drop to a glass of milk required to trigger a response. Time of onset of symptoms also varies from seconds to hours or even days.

The scratch or skin test, which is quite useful in diagnosis of inhalant allergies such as those caused by pollens, molds, animal danders, as well as some ingestants such as seeds and nuts, is unreliable in diagnosis of milk allergy. Many who are clinically sensitive to milk do not give a positive skin test and some who do give a positive test are not clinically sensitive, although a strongly positive skin test is regarded as suggestive and worthy of special attention. Diagnosis of milk allergy can only be made by evaluating several criteria. The most important test is the provocative test which consists of inducing symptoms on ingestion of milk followed by remission of symptoms on elimination of milk from the diet. This should be repeated to increase the certainty of the diagnosis. In using the provocative test non-allergic causes of symptoms such as lactose and galactose must be ruled out. This can be done by using lactose-free milk preparations for the challenge. Also, sensitivity to contaminants in the milk such as penicillin must be ruled out.

Personal and family histories, response to antihistaminics, and the passive transfer test can provide important results contributory to diagnosis.

Symptoms of Milk Allergy

The multiplicity of symptoms of milk allery and the proportions of these symptoms exhibited by patients was determined in a careful study by Goldman and associates *(18, 19, 41)* in 1963. They studied 700 pediatric patients suspected of milk allergy. A diagnosis of milk allergy was made on the basis of several criteria including principally the thrice repeated provocative test. Milk allergy was diagnosed in 89 or 13% of the patients. The patients with milk allergy were divided into two groups for the provocative tests to determine percentages of symptoms. Group A was challenged orally with 100 ml of skim milk in the form of 9 g of Starlac in 100 ml of water and with four purified milk proteins in the amounts normally found in 100 ml of milk, namely, with casein, β-lactoglobulin, α-lactalbumin, and bovine serum albumin (BSA). Group B was challenged with milk alone. The two groups reacted similarly. Multiple symptoms were produced by the challenges in 77% of the patients of both groups. The challenge symptoms and their proportions were as follows: vomiting, 33%; diarrhea, 37%; abdominal pain or colic, 28%; rhinitis, 35%; asthma, 27%; atopic dermatitis, 35%; urticaria, 11%; anaphylaxis, 9%; and central nervous system and other symptoms, 18%. When challenged orally, every patient in Group A gave an allergic reaction to one or more of the purified proteins. The incidence of reactions to these proteins was: casein, 60%; β-lactoglobulin, 62%; BSA, 52%; and α-lactalbumin, 53%. Nineteen reacted to one protein; 12 to two proteins; 7 to three proteins; and 6 to all four proteins. The median time of onset of symptoms was 1 hr with a range of 10 sec to 25 hr. The median duration of reaction was 24 hr and the range was from 40 min to 96 hr.

Symptoms attributed to cow's milk allergy are tabulated and discussed by Collins-Williams *(9)*.

Breast Milk Versus Cow Milk in Infant Feeding as Related to Milk Allergy

Milk allergy occurs primarily in infants and children up to 2 years of age. Several reasons for the allergic sensitization of infants to cow milk appear throughout the literature. From birth until the third month, the gastrointestinal mucosa is more permeable to undigested food proteins than in older children and adults. Hence feeding milk during this period exposes the sites of antibody formation to sensitizing doses of foreign protein which in some instances produces allergic antibodies, particularly in infants from families with an allergic background. It has been suggested that the infant immune system may respond more vigorously to small quantities of foreign protein than that of older children and adults. Glaser *(16)* pointed out the enormous amount of milk protein which an infant may ingest compared to an adult on a body weight basis. A 9-kg child may easily take a liter of milk daily which corresponds to about 8 liters daily for a 72-kg adult on the same body weight basis. The digestive tract is more permeable than in normal infants in certain diseases involving diarrhea which increases the sensitizing potential of ingested foods *(21)*. Likewise enzyme insufficiencies in the digestive tract can allow more undigested protein to be absorbed than would occur under normal conditions.

The question of breast feeding versus cow milk feeding of infants in the first months of life with

regard to development of milk allergy has been given much attention. Since milk allergy occurs primarily in infants and children under 2 years of age, and since milk antibodies are known to appear in infants within a month after ingestion of milk, it has seemed reasonable that breast feeding should be encouraged in the first months of life. Glaser *(16)* and others have advocated breast feeding from birth into the early months of life, and if that is not possible, a milk-free supplement or substitute should be used especially in families where an older child has milk allergy or the family has an allergic background. The views of some others have been to go ahead with a milk diet and treat milk allergy if and when it appears.

Regardless of the views of those recommending breast feeding of infants, the practice has declined markedly in the past 2 to 3 decades *(3)*. In 1948, Bain *(1)* reported that 38% of infants were entirely breast fed and 27% partially so at the time of discharge from the hospital in about 1 week. Ten years later, Meyer *(35)* reported that only 21% were breast fed and 16% partially so at the end of the fifth day. In 1963, in the Boston area, Salber and Feinleib *(40)* reported that only 22% of mothers even attempted breast feeding and nearly one-half of these discontinued in the first month.

Pertinent to this problem is the 1970 report of Gery *(15)* in which he states, "Infants fed on breast milk for different periods, before being switched to cow's milk, reacted with significantly lower levels of antibody than those fed on pasteurized or powdered milk right after birth, with the degree of reduction in response being directly related to the period of breast feeding."

Formation of Milk Antibodies

Formation of antibodies to milk proteins in the blood of infants and children following ingestion of milk is regarded as a normal immunological response *(22, 37, 41)* although there have been several reports of their association with various pathological conditions such as chronic respiratory diseases *(28, 29, 30)* and gastrointestinal illnesses *(28, 47, 48)* other than milk allergy. Milk antibodies are detectable within less than a month following ingestion of milk. The shortest reported time for appearance of milk antibodies following ingestion is 5 days *(23)*. Antibody concentration reaches a peak in about 3 months after their first appearance and gradually decreases after that *(15)*.

There are four main types of antibodies in the blood serum, I_gG, I_gA, I_gM, and I_gE which are induced by ingestion of milk. I_gG is frequently associated with anaphylactic type of reaction and I_gE is associated with the atopic allergic reactions *(31)*.

Gery *(15)* has reported that although I_gE was usually found in the serum of most cases of elevated antibody levels, including cases without allergic symptoms, in some cases of milk allergy no I_gE was found.

Although the relationship of milk antibodies to milk allergy has been studied extensively, further discussion of the subject is beyond the scope of this paper. The subject has been reviewed recently by Hanson and Johansson *(26)*.

Crib Deaths or Sudden Infant Death Syndrome

Sensitivity to milk has been suggested as one of many possible causes of sudden and unexpected death in infants, commonly known as crib deaths in the United States and cot deaths in England. The common-usage term "crib death" will be used in this paper instead of the scientific term "sudden infant death syndrone" *(49)*. This subject has been excellently reviewed by Valdes-Dapena *(50)*.

Crib deaths in the United States account for 15,000 to 25,000 human lives annually *(50)*. Parish and associates *(36)* reported in 1955 that there were 1432 crib deaths in England and Wales. Carpenter and Shaddick *(7)* estimated that these deaths accounted for 16% of all infants deaths in England and Wales in 1960. Emery *(11)* suggested that the number of crib deaths is increasing in Great Britain each year. Fontaine in 1962 reported that in France crib deaths accounted for 10% of all infant deaths in 1954 *(12)*. It is apparent that crib death is a major health problem. It is generally agreed that the greatest number of crib deaths occurs in infants under 6 months of age with the peak incidence between 2 to 4 months.

The usual history of crib death is that a well child or a child with a minor illness is fed and put to bed. Within the next 3 to 4 hr the infant dies without struggle or noise. The psychological trauma of this on parents is devastating. And, in the United States, there are two organizations composed of parents who have experienced this tragedy to help them cope with its aftermath *(49)*.

Explanation of the cause of crib death is controversial. Some believe that a single mechanism is involved in most cases while others believe there are many causes. Only 15% of the deaths can be satisfactorily explained on autopsy. The possible causes of the deaths are discussed in detail by Valdes-Dapena *(50)*.

A few of these causes are suffocation by overlay or bedclothes, fulminating viral or bacterial infections, and hypersensitivity to cow milk. It is of interest to describe briefly the development of the milk allergy hypothesis and some of the experimental evidence supporting it.

In 1954, Barrett *(2)* in England, suggested that crib deaths might be caused by inhalation of an amount

of food too small to cause asphyxiation *per se* but which might cause an inflammatory reaction with edema which could cause death. In 1960, Parish and associates *(36)* hypothesized that since milk antibodies can usually be detected in infants that at least in some cases, crib deaths may result from anaphylactic-type shock on inhalation of milk proteins in vomitus. These authors simulated crib death in model experiments using normal guinea pigs and those sensitized with cow milk protein. Introduction of up to 2 ml of soluble milk proteins into the respiratory tract of an unsensitized guinea pig produced negligible effects, whereas introduction of this amount into sensitized guinea pigs produced severe, typical anaphylactic reactions often terminating in death. If, however, only 0.25 ml of milk or gastric contents from an infant who died of "cot death" were introduced into the upper respiratory tract of a slightly anaesthetized, milk-sensitized guinea pig to simulate sleep, many animals died rapidly without struggle similarly to crib death in infants.

Experiments of Parish and others aroused great interest and research has been continuing, especially in England. As usual there are investigators who challenge the foregoing explanation of crib deaths *(17)*. It is evident that because crib deaths are a major health problem and because milk has been implicated, that further research should be done to clarify the role of milk in this connection.

Milk Allergens

Milk proteins are the etiological agents in milk allergy. Almost all proteins are antigenic and, while not all antigens have been characterized as allergens, nevertheless, they must be considered as potential allergens until definite information is available.

Milk is a very complex mixture of proteins consisting of the caseins and the whey proteins. Among the known caseins are the genetic forms of α_{s1}-casein, β-casein, *k*-casein, and γ-casein. The whey proteins consist of the genetic forms of β-lactoglobulin and α-lactalbumin. BSA, the immunoglobulins, and several enzymes are also present in whey. The allergenic activity of β-lactoglobulin, α-lactalbumin, BSA, and casein have mainly been studied. Allergenic activity has been attributed to all of these proteins. Although, principal allergenic activity has been ascribed to β-lactoglobulin and α-lactalbumin, the question is controversial. One difficulty in assessing allergenic activity of so-called pure proteins is that almost all preparations are contaminated with other milk proteins and allergenic activity can be triggered by minute amounts of a protein.

The immunoelectrophoretic technique has been very useful in demonstrating at least a minimum number of separate antigens in milk. Hanson *(24)* and Hanson and Johansson *(25)* have shown at least 12 to 14 separate antigens in mature bovine milk with many more in colostrum which come from blood serum.

Berrens *(4)* has postulated and presented considerable though not conclusive, evidence that a carbohydrate-lysine linkage (lysyl-[ε-amino-(1)]-1-deoxy-2-ketose) formed in the browning or Maillard reaction is a grouping responsible for atopic allergic reactions. Bleumink and Young *(6)* reported over a 100-fold increase in skin reactivity on milk-sensitive persons to relatively inactive β-lactoglobulin after prolonged heating with lactose at 50 C at pH 7.0. They attributed this increased skin-reactivity to a browning reaction condensation product of lactose and the ε-amino group of lysine in the β-lactoglobulin. These authors did not use a control test for non-dialyzable antigens in the lactose. This point was tested by Spies *(42)* who determined that two lactose samples contained small amounts of 4 new antigens in the retentate which were not identifiable with known milk proteins. Therefore the increased skin reactivity attributed to the browning reaction product of β-lactoglobulin and lactose may be due to these new antigens in lactose. The point requires clarification.

Prognosis

Milk allergy, which starts in the first months of life, fortunately disappears relatively early in life. Clein *(8)*, who reported 6% incidence of milk allergy in his pediatric practice, stated, "Somewhat less than 80% of infants lose their allergy to cow's milk before they become a year old. Somewhat less than 15% more lose their allergy to cow's milk by the time they are two years old. About 2% of the infants who initially had allergies to cow's milk continue to be allergic to this food after they reach the age of six years." However, Clein stated that about 80% of the infants who had cow milk allergy develop major allergies to other things before the age of puberty. Several authors believe that if milk allergy can preferably be prevented or at least treated promptly, it would tend to minimize the chances of milk allergic infants developing other allergies in later life *(16, 32)*.

Current Work on Milk Allergens at the Dairy Products Laboratory

The broad objective of the allergens investigations of the Dairy Products Laboratory of the Department of Agriculture is the control or inactivation of the allergens of milk so that this nutritious food will be acceptable to those who cannot now tolerate it because of allergic response to ingestion of milk. The relatively low incidence of milk allergy in the total

population (<0.1 to 7%) might seem too insignificant to justify research on milk allergy by Dairy Products Laboratory. However, the significance of this work is broader than these figures indicate at first glance. Firstly, it is pediatric practice in many cases in families with a history of any allergy to eliminate milk from the diet of infants and children under 2 years of age whether they are allergic or not to minimize their chances of acquiring milk allergy. Secondly, as pointed out above, it is believed that prevention of milk allergy in infants tends to lessen the chances of infants acquiring other allergies in later childhood, after milk allergy, should they have acquired it, had disappeared. And thirdly, milk contains well-characterized proteins which are ideally suited as model substances for studying all food allergies.

It must be evident from the foregoing brief review of the subject of milk allergy that the present state of knowledge requires, primarily, fundamental scientific investigations to elucidate the mechanism of the allergic response to ingestion of milk before we can elaborate feasible procedures for the control of milk allergens.

For the past 5 years we have been studying the immunological significance of pepsin hydrolytic products of milk proteins. The immunologic significance of enzyme hydrolytic products of ingested allergenic proteins long has been the subject of speculation and sporadic investigations. The consensus of the clinical significance of digestive products has been that they may be the cause of delayed clinical reactions of from 1 to 36 hr or even days in some instances. Our work suggests that digestive products of milk may be the cause of immediate type allergic response possibly in addition to delayed responses.

We first demonstrated *(46)* generation of a new antigen in the dialysate of the 8-min pepsin hydrolysate of each of 4 major milk proteins, namely, β-lactoglobulin, α-lactalbumin, casein, and BSA. The endo fraction of BSA contained a second new antigen. In our studies the term "new antigen" is defined as an antigen with a specificity distinct from that of the protein from which it was generated.

The objective of later studies *(43, 44)* was to determine whether one or several new antigens are generated by a simulated stomach digestion of β-lactoglobulin. In this study β-lactoglobulin was hydrolyzed six, successive, 8-min periods during which approximately 90% of the protein was split into fragments with a molecular weight of 12,000 or less. Six dialysate (D1-D6) and six endofractions (E1-E6) were separated and analyzed for the presence of new antigen using the Schultz-Dale and gel diffusion analysis, respectively. All of the dialysates contained common, nonprecipitating new antigens. The first dialysate (D1) did not contain all of the new antigens common to the other five, D2-D6, indicating at least two new antigens in the dialysates. Six precipitating new antigens were demonstrated in the endofractions. Now by anology to β-lactoglobulin, if pepsin hydrolysis generated at least eight new antigens from each of the 12 to 14 antigenic proteins in milk, the body immune system would be exposed to about 100 new antigens, all of which are potential allergens, on ingestion of milk. These results may explain why milk and other foods, in many instances do not give skin reactions on persons who give an immediate allergic response on ingestion of the food. Such persons may be sensitive to these new antigens formed by pepsin in the stomach during digestion.

Figure 1. Demonstration of common new antigen in dialysate fractions, D1 and D2, and that D2 contains new antigen other than that present in D1 by Schultz-Dale technique. Sensitizing antigen: D2. Challenge doses in μg total nitrogen of fractions: M(β-lactoglobulin, pepsin, PEPD), each component, 10: D1, 10: D2, 10.

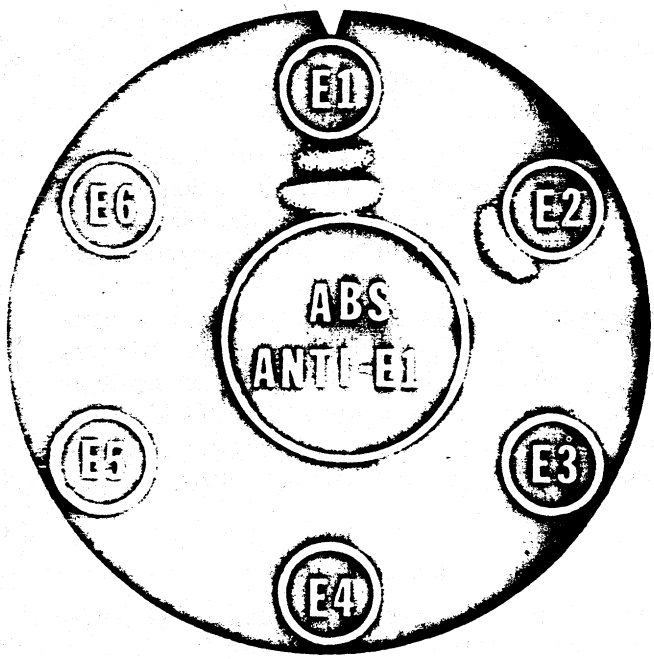

Figure 2. Demonstration of precipitating new antigens in endofractions, E1 and E2, by gel double diffusion analysis. Center well, 0.5 ml of absorbed anti-E1 (rabbit) (absorbed with β-lactoglobulin and PEPE); peripheral wells, 0.07 ml of indicated endofraction, 0.5 mg endofraction nitrogen per ml.

Although the sensitizing properties of these new antigens are unknown as yet, it seems likely that some of them, at least, might act as allergic sensitizers for food digestion products in a manner similar to that of other low-molecular weight substances such as drugs. It was demonstrated *(46)* that new antigen could be detected after only 1, 2, and 4 min pepsin hydrolysis of total milk protein. Later *(43)* it was apparent that common new antigens continue to be generated over a period of 48 min. Since absorption of immunologically significant amounts of allergens are known to occur in a few minutes *(27, 45, 52, 53)*, this continuous production of new antigens in the dialysates tends to enhance their sensitizing potential.

The Schultz-Dale technique, was used to demonstrate the two, nonprecipitating new antigens in dialysates, D1 and D2, as shown in Fig. 1. Figure 2 illustrates use of the gel double diffusion technique in demonstrating two precipitating new antigens in endofraction E1. Details of these studies are described in reference *43*.

Current studies are in progress on isolation and chemical and immunological characterization of these new antigens. Following this the purified fractions will be evaluated clinically to determine their allergenicity.

Demonstration of this multiplicity of new antigens (potential allergens) generated by pepsin hydrolysis of milk proteins, as a simulated first step in digestion, opens up a new area of study which should clarify an important aspect of the many perplexing aspects of food allergy in general and milk allergy in particular.

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